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(54) Title: RADIOACTIVITY LOCAL DELIVERY SYSTEM

(57) Abstract

The invention provides a radioactivity local delivery system enabling the use of isotopes having a longer half life and having a lower energy. The invention also provides a radioactivity local delivery system enabling an easier and more efficient control of the dose/rate and total dose of local radiation delivery. The present invention utilizes a radioisotope coupled to a circulation time reducing agent such as a chelatin agent, the coupled radioisotope being in releasable association with the structure to be inserted in a vessel of a human patient. The coupled radioisotope can be part of the support itself or in releasable association therewith through the use of a biodegradable or non-biodegradable pharmaceutical carrier. Also provided is a method to decrease the growth of actively proliferating cells at a targeted site in a vessel of a human patient as well as compositions therefor.

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## TITLE OF THE INVENTION

RADIOACTIVITY LOCAL DELIVERY SYSTEM

## FIELD OF THE INVENTION

The present invention relates to the local delivery of radioactivity. The present invention further relates to the localized inhibition of cell proliferation using radioactivity.

## BACKGROUND OF THE INVENTION

The therapeutic use of radiation therapy to reduce the proliferation of rapidly dividing cells has evolved from the Bergonié and Thibondeau Law of radiobiology which states that proliferative cells are more radiosensitive than normal cells (Bergonié and Thibondeau, 1959, Radiat. Res. 11:587). Hence, radiation therapy can be used to reduce proliferation cells in a tumor. The Bergonié and Thibondeau principle need not be limited to the treatment of malignant tumors, however. A number of clinical situations require the reduction of cell proliferation: treatment of heterotopic bone formation, prevention of cheloids and more recently the inhibition of intimal hyperplasia. Having discovered that smooth muscle cell proliferation is inhibited after irradiation, radiation therapy has thus been applied to reduce the restenosis process following coronary angioplasty.

Coronary angioplasty is actually a well established technique for the treatment of obstructive coronary disease. More than 500,000 angioplasties are performed every year world-wide. However, two major problems remain unsolved. The first is acute closure, reported to occur in up to 11% of the cases after balloon angioplasty (Dorros et al., 1983, Circulation 67:723-730). In that context, intracoronary stenting appears as an invaluable procedure for the treatment of extensive dissections occurring after angioplasty. As a scaffolding vessel wall

support, it preserves adequate coronary opening and perfusion. The second problem is restenosis which has been shown to occur in 30 to 50% of the cases. So far, drug therapy has shown limited results in reducing the extent of the phenomenon (Popma JJ et al., 1991, Circulation 84:1426-1436). Intracoronary stents have been shown in randomized trials, to reduce restenosis from 42% to 32% in the Stress trial while from 32% to 22% in the Benestent trial (Fischman DL. et al., 1994, N. Engl. J. Med. 331:496-501; Serryus PW. et al., 1994, N. Engl. J. Med. 331:489-495). The beneficial effect of stenting is presumably due to a better vessel geometry after dilation, although stenting has been proved to induce more neo-intima formation than other devices in swine (Karas SP. et al., 1992, J. Am. Coll. Cardiol. 20:467-474). Indeed, swine has been recognized as a relevant animal model for restenosis although the rat and rabbit animal models are also widely used. Morphologically and hemodynamically the porcine coronary vasculature is very similar to the human coronary system. Reproducible intimal proliferation is obtained after balloon injury in the pig coronary arteries. Histologically, the proliferative response to balloon injury in the pig coronary is very similar to the response seen in pathological studies of humans (Schwartz et al., 1990, Circulation 82:2190-2200).

Balloon dilation leads to global vascular lesions which include mechanical deformation of the vessel, extensive destruction of the endothelium and immediate formation of thrombus. All of these act through vasoactive hormones, growth factors, circulating cells and presumably lipids on the media muscle cells. It is observed that smooth muscle cells are activated and migrate to the intima where after proliferation and matrix secretion, a "neo-intima" is generated [Hamon et al., 1995, Eur Heart J 16(Suppl 1):33-48]. This observation led to the proposal of a cellular mechanism for restenosis (figure 1). The role of elastic recoil and vessel remodelling has also been recognized following

angioplasty (Kakuta T. et al., 1994, Circulation 89:2809-2815). Finally, thrombus adhesion through growth factors liberation, also plays a major role in the activation cascade (Fager G. et al., 1995, Circ. Res. 77:645-650).

Until now, drug therapy has consistently been focused on proliferation and thrombus inhibitions (Popma JJ et al., 1991, Ibid.). Unfortunately, no significant effects were observed in human coronary restenosis when the drugs were administered systemically (Popma JJ et al., 1991, Ibid.). The lack of a sufficient local drug concentration is the most common advocated reason to explain the inability to reduce neointima formation in humans. The research has thus targeted the local delivery of different drugs to prevent restenosis (Lincoff AM et al., 1994, Circulation 90(4):2070-2084). New catheters are already available to deliver drugs locally after angioplasty and feasibility trials are underway (Fram DB et al., 1994, J. AM. Coll. Cardiol. 23:186A).

Although, the genetic and molecular understanding of the different mechanisms involved in restenosis have also been greatly improved, due to its complexity, the clinical genetic treatment of restenosis is expected to be very expensive and not readily available for still some time (Bennet MR. et al., 1995, Circulation 92:1981-1993).

The use of radiotherapy to reduce neointima formation was thus identified as a possible solution to the restenosis problem. Three basic approaches utilizing radiotherapy have thus been opposed:

**1) External irradiation:**

External delivery using Gamma or Beta irradiation, showed that at the single high doses used, a decrease in hyperplasia is observed. However, some groups detected fibrosis or necrosis in the irradiated region (Schwartz RS. et al., 1992, J. Am. Coll. Cardiol. 19:1106-1113). Moreover, this type of approach encompasses the irradiation of a large field.

**2) Radioactive catheter**:

Experiments carried with endovascular irradiation at the high dose/rate of Beta or Gamma rays produced a significant reduction in neointimal formation. However, this positive effect seems to be accompanied by fibrosis of the vessel resulting in a loss of vasculator function thereof, suggesting that in the long term such a type of treatment might be detrimental. Furthermore, the irradiation treatment at the time when the proliferation potential of the smooth muscle cells is that peak (i.e. 24 - 48 hours) would be at best impractical in a clinical situation. Moreover, arteries receiving higher doses showed an increase diameter suggesting that irradiation would affect vessel remodeling [Waksman R. et al., 1995, J. A. Coll. Cardiol. (Special Issue (February 95)]. Of note, Brenner et al., 1996 (Radiation Oncology Biol. Phys. 36: 805-809) showed that a single high dose does not inhibit restenosis. Also, it has been reported that a 18 Gy single irradiation, failed to show a significant reduction in restenosis.

**3) Radioactive stents**:

The group of Fischell studied the effects of $P^{32}$ stent wire on smooth muscle cells and endothelial cells proliferation in tissue culture (Fischell TA. et al., 1994, Circulation 90:2956-2963; and US patents 5,059,166 and 5,176,617). Titanium wire which was first impregnated with $P^{31}$ and then activated in a fission reactor was used. The resulting radioactive stent is thought to be emitting Beta radiation, although contaminating $\alpha$ and $\gamma$ emissions are likely because of the impregnation method. The use of such a stent on muscle cells demonstrated a dose response curve of inhibition at linear activities. However, at the highest wire activity level, there was inhibition observed as far as 10.6 mm from the wire.

This degree of penetration suggests that the stent emits Gamma rays and that the use thereof *in vivo* would not deliver the

radiation specifically to the targeted site, since a significant amount of normal surrounding tissue would be irradiated. This issue, amongst others, was indeed raised by Crocker et al., 1995 (Circulation 92:1353). The ion implantation technique creates lattice defects in the metallic crystal structure resulting in stoichiometric modification. These defects can contribute to diffusion of ions (leeching) modification of surface potential and alterations in clinical properties. Consequently, this method of radioactivation can alter the biocompatibility of the stent surface and hamper human clinical use.

Radiotherapeutic treatment such using radioactive stents showed a significant reduction in neointima formation that was dose-dependent, it also suggested a delayed regeneration of endothelial cells. Together with the long-range irradiation of surrounding tissues, this type of stent can be foreseen as having detrimental effects, especially in the long term, on the integrity and functionality of the treated vessel and surrounding tissues.

As mentioned previously, a number of animal model have been used to assess the feasibility and elaborate the methods of radiation therapy to be applied to humans. Radiotherapy of cancer is currently routinely used in humans. However, local delivery of radioactivity has yet to show its full potential. Only a few studies on radiation therapy following angioplasty in humans have been performed. All experiments dealt with relatively high dose rates. Using $Ir^{192}$ Gamma irradiation source which delivered 2000 cGy in durations lasting from 5 to 15 min [Condado JA. et al., 1995, J. Inv. Cardiol., 1995, 7(SupplC):25C; Condado JA. et al., 1995, J. AM. Coll. Cardiol., 1995, (Special Issue (February 95):288A], mild spasms occurred in the majority of the treated coronary arteries. However, with the group which received 2500 cGy, 7 out of 8 treated arteries developed aneurisms. In the group with

2000 cGy, out of 12 treated arteries 4 developed restenosis. Thus, side effects with significant potential health hazard were recorded.

The feasibility of radiation therapy to inhibit cellular proliferation is suggested by increasing data on the relative resistance of non-actively proliferating cells versus their actively proliferating counterparts. The resistance of non actively proliferating cells to radiation treatment is only relative, however, as assessed by the inhibitory effect of radiation on endothelial cells, or the fibrosis or other side effects promoted by the radiotherapy.

Pure Gamma, pure Beta and mixed irradiations have been tested. Since the thickness of the arteries is in the mm range, Beta-irradiation is preferred over Gamma-irradiation in angioplasty-related applications, due to the known deeper effects of the latter (Waksman R. et al., 1995, Circulation 92:1383-1386). Obviously, Beta rays also present advantages concerning radioprotection. However, due to the limitations associated with the fixing of the isotope onto the support such as a stent (i.e. suitable isotopes, shelf life of the radioactive stent, production costs, etc.), the use of a Beta-isotope in such systems does not offer an optimal solution.

The question of half-life of the isotope used is of crucial importance from a practical, as well as from a biological point of view. It is generally understood that it is preferable to choose an isotope that would irradiate for the minimum while sufficient time required to inhibit the proliferative activity of the targeted cells, thereby minimizing the irradiation of the surrounding tissues. It is known that in order for less than 1% of the total radioactivity of an isotope to remain requires the passing of 6 half-lives. However, the removal of the remaining 1% radioactivity will require a very long time if not an infinite amount of time. In the case of 32P for example, which has a half life of 14 days, 84 days are required to bring the level of radiation below the 1% mark. In the

treatment of restenosis, for example, as radioactivity will mainly target its global effector, namely, smooth muscle cells proliferation, the need to be effective throughout the replicative stage (approximately 15 days) have to be taken into consideration. Thus, a significant proportion of the radioactivity will remain in place long after the proliferation stage of the smooth muscle cells. It follows that the current technology is limited since the total radiation dose is controlled solely by the half life and quantity of the chosen isotope.

It should be noted that all systems designed at delivering radioactivity to a targeted region have been based on implantation of the radioisotope to or beneath the surface of a support such as a stent. These systems aim at minimizing or even abrogating any "leeching" of the radioactivity from the support (Fischell et al., 1995, Circulation 92:1353-54). It should also be understood that to date, no local radiation delivery system permits a flexible calculation and control of the total dose in the patient.

It would be beneficial for the medical and research practitioners to be provided with a radioactivity local delivery system that would be more practical to use, would limit unnecessary exposure to normal surrounding tissues, and could permit a more precise control of the dose and the dose/rate of irradiation of the targeted cells.

## SUMMARY OF THE INVENTION

A first aim of the present invention is to provide a radioactivity local delivery system which obviates the drawbacks of the prior-art.

A second aim of the invention is to provide a radioactivity local delivery system which permits an optimization of the radiatiotherapeutic treatment.

Another aim of the present invention is to provide a radioactivity local delivery system which permits the use of isotopes

having a longer half life. As well, the present invention permits the use of radioisotopes with low energy.

Another aim of the invention is to provide a radioactivity local delivery system which permits a control of the total dose of local radiation delivery.

An additional aim of the present invention is to provide a radioactivity local delivery system which permits an optimization of the duration of local radiation delivery.

A further aim of the invention is to provide a radioactivity local delivery system which enables a control of the efficacious dose/rate for inhibiting cellular proliferation.

Yet another aim of the invention is to provide a radioactivity local delivery system which eliminates the potential biocompatibility and hemocompatibility problems associated with particle bombardment of a stent or stent-like structure aimed at rendering same radioactive.

As well, a further aim of the invention is to provide a radioactivity local delivery system which will also enable the delivery of one or more drugs or biological agents.

In accordance with the present invention, there is provided a radioactivity local delivery system comprising: a) a support of generally tubular structure having an external surface adapted to engage the wall of a vessel of a human patient; b) a radioisotope in releasable association with the support; and c) a chelating agent coupled to the radioisotope, whereby upon placement of the radioactivity local delivery system inside the vessel, the releasable association between the support and the radioisotope enables a release thereof into the circulation of the human patient at a rate controlled by the rate of release of the association, and the chelating agent enables a rapid elimination of the radioisotope from the circulation.

In accordance with the present invention, there is also provided a kit comprising a support of generally tubular structure having an external surface adapted to engage the wall of a vessel of a human patient and a radioisotope coupled to a chelating agent, wherein the radioisotope is in releasable association with the support for locally delivering to a targeted site of a human vessel a predetermined dose of radiation.

In accordance with the present invention, there is also provided a cell proliferation inhibiting composition comprising a radioisotope coupled to a, chelating agent wherein the radioisotope is in releasable association with a pharmaceutically acceptable carrier, whereby upon placement of the cell proliferation inhibiting composition at a targeted site in a human patient, the irradiation by the radioisotope inhibits a proliferation of actively proliferating cells and whereby the releasable association between the radioisotope and the carrier enables a controlled release of the radioisotope from the targeted site at a controlled rate into the circulation of the patient and the chelating agent enables a rapid elimination of the radioisotope from the circulation.

In accordance with the present invention, there is an addition provided a method to decrease the growth of actively proliferating cells at a targeted site in a vessel of a human patient comprising the insertion and positioning at the site of a composition comprising a radioisotope coupled to a chelating agent, wherein the radioisotope is in releasable association with a pharmaceutically acceptable carrier; such that irradiation will inhibit a proliferation of the actively proliferating cells and such that by way of the releasable association between the radioisotope and the carrier, the radioisotope will be removed from the targeted site at a controlled rate into the circulation of the patient and the chelating agent enables a rapid elimination of the radioisotope from the circulation.

## BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a simplified model of the restenosis pathway.

Fig. 2 is a schematic representation of the exposed tissues using a low energetic radiation; (A) cross sectional view of the vessel with stent in position; (B) cross sectional view along line 1.

Fig. 3 is a schematic representation of a frontal view of a Wiktor stent with six radioactive coated struts.

Fig. 4 (A) is a schematic representation of a side view of the Wiktor stent with a wire wrapped around it; Fig. 4 (B) shows the wire unwrapped and stretched.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention therefore aims at controlling cell proliferation through local isotope irradiation. It should be understood, herein, that the present invention is not limited to a use in the reduction of coronary restinosis. Indeed, it is contemplated that the present invention can be used in a variety of clinical situations that include, without being limited thereto, cancer therapy, inhibition of keloide scares and treatment of heterotopic bone formation. The medical practitioner will be able to adapt the present invention to a particular clinical situation. For example, placement of a stent-like structure in a duct or track or even in bronche could locally deliver radiation, thereby inhibiting the proliferation of the targeted cells. It should understood that the present invention can be adapted by a medical practitioner to enable modification of cancer radiotherapy of a chosen tissue or organ by the use of the present invention.

In one preferred embodiment, the present invention is aimed at controlling smooth muscle cells proliferation with a radioactive stent to reduce coronary restenosis after balloon angioplasty. Many

arguments suggest the effectiveness of local radioactivity to reduce neointimal proliferation after balloon angioplasty. Coronary stents are increasingly used after angioplasty and indications will probably broaden in the future. The combination of both strategies in some clinical circumstances has previously been disclosed by the group of Fischell and others. However, the disclosed methods rely on a stent rendered radioactive by activating the metal of the stent with particle bombardment (reactor, cyclotron). In that case, different radioisotopes emitting Gamma and Beta radiations with several energies and half lives can be produced (Herlein C. et al., 1995, Circulation 92:1570-1575). It should be stressed that it remains to be determined whether the biocompatibility and hemocompatibility of the stent has been modified by the process of rendering it radioactive.

The present invention overcomes the drawbacks of the Fischell strategy by providing a system which can enable a precise control of the dose and dose/rate of irradiation. One such system which is provided consists in putting an available isotope on the stent itself. One way to reversibly fix the isotope is to mix it with a polymer or other carrier that would be bonded on the stent. By providing a polymer or carrier, the degradation thereof will enable the irradiation to be controlled by the rate of degradation of the substance and the half life of the isotope itself, thereby providing a significant increase in the flexibility of the radiation delivery system. In the example of a stent coated with a polymer or carrier into which the isotope would be mixed, after positioning in the artery following angioplasty, the polymer-isotope composition can be chosen so that irradiation will be present during the proliferative activity of the smooth muscle cells and absent thereafter. It is to be understood that the polymer need not necessarily be biodegradable. However, the polymer must provide for a controlled release of the radioisotope mixed therein.

Also contemplated in the present invention is the integration of the radioisotope inside the structure of the polymer or carrier forming the stent or support structure itself. In other words, in such an embodiment, the stent or support structure would be both biodegradable and radioactive. Biodegradation thereof would thus play the dual role of eliminating the radioactive source from the targeted area as well as removing the support structure therefrom. It is also conceivable that in certain clinical situations, a stent or support could be withdrawn after a predetermined time.

The coupling of the radioactivity isotope in accordance with the present invention with an agent enabling its rapid elimination from the circulation is at the crux of the invention. Such a combination enables a minimization of the potential hazard of irradiation at distant sites from the chosen sites of local radiotherapy. Maximum complex stability is achieved via chelation (Cotton and Wilkinson 1980). A chelate is formed when a metal atom is bound to more than one donor atom of a complexing molecule or ligand, thereby forming a closed-ring structure. The added stability of the metal chelate is often required in order to resist oxidation, hydrolysis or the strong affinity that some metals (especially indium and gallium) display for the plasma protein transferrin. Diethylene-triaminepentaacetic acid (DTPA) was on of the first chelating agents to be used to complex $^{99}Tc^{m}$ (Richards and Atkins 1968). Tc-DTPA is primarily excreted from the body via the kidneys and is still used today to assess renal function. At least a dozen Tc complex have been described that demonstrate renal accumulation and/or clearance (Eckelman and Volkert 1982). In general, the metal-chelate complexes have characteristic physicochemical properties (i.e. molecular weight, lipid solubility, charge $pK_a$ proportion of protein binding, etc.), which will govern their *in vivo* distribution (Webb Ed., 1993, The Physics of Medical Imaging, Inst. of Physics Publishing, Bristol and Philadelphia). It will be

understood that the chelating agent will be dosed so as to promote the execution of the radioisotope following leeching from the carrier or support.

The concept of coating a stent or support structure with a polymer has been described several years ago and is discussed in the literature regularly. The local delivery of drugs is centred on two concepts: a direct coating of the stent or support structure with a drug or drug carrier combination or the incorporation of a drug into the stent or support structure, the stent or support structure being constructed of a biodegradable polymer. The adaption of this drug delivery concept to radiation delivery is novel, as all previous attempts and focus have been focused on the implantation of the radioisotope to an inert substance and/or in such a way as to ensure that no leeching of radioactivity occurs. Recently, another type of drug delivery system has been disclosed in U.S. 5,383,928. A sheath as disclosed therein could also be adapted so as to provide a local delivery of radioisotope in accordance with the present invention.

Based on the above and the demonstrated effects of radiation on the proliferation of cells, it would be expected that the radioactivity local delivery system would significantly reduce neointima formation following angioplasty and proliferation of actively proliferative cells such as for example tumor cells. Once again it will be understood that the medical practitioner can adapt the dose and dose/rate so as to for the treatment to a cancer therapy. Similarly, the type of isotope, carrier and the like will be adapted to meet the desired needs.

The separate sleeve which encompasses the stent and can also serve as a local drug delivery device to prevent restinosis. When combined with a drug, the separate sleeve can further prevent thrombosis and/or restinosis. It should be understood that such a sleeve need not be limited to a use with a stent designed for coronary

angioplasty. Indeed, such a sheath can be used with a stent to deliver radioactivity to an arterial wall or lumen, vessel, duct or passage in which the stent has been inserted.

Herein, the term "vessel" is used broadly to cover lumen, duct, and other types of bodily conduits.

By "drug" is meant any compound which has a desired pharmacologic effect. Naturally, the drug is compatible with the polymer and can be tolerated in a subject. For example, the drug can be an anticoagulant e.g. D-Phe-Pro-Arg chloromethyl ketone, and RGD peptide-containing compound, heparin, antithrombin compounds, platelet receptor antagonists, anti-thrombin antibodies, anti-platelet receptor antibodies, aspirin, prostaglandin inhibitors, or platelet inhibitors. The drug could also be a promoter of vascular cell growth, e.g. a growth factor inhibitor, growth factor receptor agonist, transcriptional activator or translational promoter. Alternatively, the drug could be an inhibitor of vascular cell growth, e.g. a growth factor receptor antagonist, transcriptional repressor, translational repressor, antisense DNA, antisense RNA, replication inhibitor, inhibitory antibodies, antibodies directed against growth factors, bifunctional molecules consisting of a growth factor and a cytotoxin, bifunctional molecules consisting of an antibody and a cytotoxin. Selected examples of days which have been used to inhibit restenosis is include methylprednisome, colchicinie, forskoline, vitamin A, and anti IIb / IIIa antibodies. In addition, the drug could be a cholesterol-lowering agent, a vaso-dilating agent, and agents which interfere with endogenous vasoactive mechanisms, anticancer agents, chemotherapeutic agents, and agents which selectively affect actively dividing cells.

Polymers which can be used as carriers in accordance with the present invention include without being limited thereto natural biodegradable polymers such as fibrin, synthetic biodegradable polymers

comprising polyglycolic acid/polylactic acid, polycaprolactone, polyhydroxybutyrate, polyorthoester, polyethyleneoxide/polybutylène terephtalate, and non biodegradable polymers such as polyurethane, silicone and polyethylene terephtalate. Of course, mixtures of polymers could also be used.

The methodology to obtain the radioactive stent has been elaborated taking in account radiobiological data concerning dose-rate and tolerance-dose. With that preoccupation, compromises in regard to radiation-type, emission-energy and exposure-time have been determined.

In restenosis, smooth muscle cells proliferation occurs in a thin layer of about 400-800 µm of the inner vessel wall. For this particular application, β-irradiation has been put forward. Indeed, as compared to γ-irradiation, β-irradiation has a much lower tissue penetration. As a consequence, a β-emitter has been selected for its energy spectrum, based on the depth of tissue over which radiation has to be applied. β-emitters having half-lives of between 1 to 1000 days are contemplated in the present invention in relation with restenosis inhibition. As mentioned above, β-emitters having a modified structure which enables them to be rapidly eliminated from the circulation are contemplated. A chelated isotope is one of many such examples of an isotope modified so as to be rapidly eliminated. A specific example of such a chelated isotope is $^{45}$Ca EDTA (calcium). Delating groups encompassed by the present invention include ethylene diaminetetraacetic acid (EDTA) and diethylene triaminepentaacetic acid (DTPA).

Preferably, for restenosis inhibition, the chosen isotope is a pure β-emitter. One such non-limitative example is $^{35}$S (sulfur), with a linear energy spectrum with a principal (maximum) energy of 167.4 KeV and an average energy of 48.8 KeV (Cross WG. et al., 1983, Phys. Med.

Biol. 28:1251-1260). The principal β particle can travel over a distance of about 400 μm in biological tissue (Dutreix A. et al., 1982, Dosimétrie en curiethérapie, Masson, Paris, New York). This spatial range seems adequate for the targeted vessel wall layer of smooth muscle cells (≈ 400 μm). Moreover, as will be presented, this range of action (maximum energy) reveals itself to be a good compromise for the intended exposure duration and dose tolerance limits. Another advantage is that this substance is commercially available and has a current medical usage in the form of sodium sulfate injection for the measurement of extracellular fluid volume. As a consequence, there is a medical history for this substance with known dosage, biodistribution and side effects (Iturralde Mario P., 1990, Dictionary and Handbook of Nuclear Medecine and Clinical Imaging, CRC Press, Boston). This substance mostly affects cartilage, indeed in the cited example, with an administered activity of 100 μCi, there is a dose of 1.9 mGy to the cartilage, 0.5 mGy to the bone marrow and 0.03 mGy to the whole body. Finally, the physical half life of $^{35}S$, which is 87.9 days presents certain logistic advantages concerning surgery planning, storage and other time delays. As discussed below, the biological preoccupation of time exposure are taken into account with the biodegradation time of the polymer matrix used to fix the isotope to the stent in our preferred embodiment. The same principle can be applied to the incorporation of the isotope in another type of carrier such as a gel or sheath or for the incorporation of the isotope in the stent or support itself. This approach presents the double advantage of having a radioactive stent with a practical physical life span and an appropriate biological duration.

The isotope can be embedded into a polymer matrix fixed to the stent. The polymer can be an organic or an inorganic polymer. As mentioned previously, non-limitative examples of organic and inorganic polymers include fibrin and PLLA/PGLA, respectively

(Tanguay et al., 1994, Cardiology Clinics 12:699-713). The rate of degradation of the polymers can control the duration of the radiotherapy. As previously mentioned, this approach allows to determine the total dose according to a selected effective and safe dose/rate. As presented in the following lines, this dose/rate and total dose are deduced from current values used in radio-oncology procedures. As discussed below, a period of 20 days is considered in the dosimetry calculations. Generally, a period of 5 to 60 days is contemplated herein. For restenosis applications, preferably 10 to 30 days and more preferably 20 days are contemplated. It should be understood that this 20 days of irradiation can be adjusted to take into account a particular clinical situation, and is thus only one example of a chosen time of irradiation. In a first approach, this time range appears reasonable in view of the fact that proliferation of smooth muscle cells occurs during a period of approximately two weeks. High dose/rate is defined herein as dose/rate equal or superior to 1 Gy/min and low dose/rate as dose/rates below 1 Gy/min.

For radiotherapy in accordance with the present dose/rate invention of 0.0-5 Gy/hr - 0.1 Gy/min, preferably about 0.1 Gy/hr - 0.5 Gy/hr are contemplated. Total doses of between 20 and 200 Gy are contemplated. A dose of 60 Gy in 7 days equivalent is contemplated. Without being limited to particular hypothesis, it would appear that the present invention by providing an irradiation at lower doses enables a significantly selective killing of actively dividing cells (such as cancer cells or smooth muscle cells) as opposed to slower dividing cells. This selectivity is explainable by the DNA repair system in the slower dividing cells being able to correct the DNA defects prior to cell division when low dose/rate or fractionated radiation is used. Since radiation affects all cells of the vascular wall, late effects could be related to the delayed depletion of some cells (adventitial cells, fibroblasts) with subsequent overshoot or repopulation. Smooth muscle cells from the

media could be progressively replaced by fibroblasts and extracellular matrix (fibrosis) and progressive cellular depletion of the vascular wall could lead to aneurysm formation.

Non-limiting examples of pure β-emitters include strontium/Itrium$^{90}$, Erbium$^{169}$ and Prometheum$^{147}$. A non-limiting example of γ-emitters includes Iodine$^{125}$. Mixed β- and γ-emitters are also contemplated as encompassed within the scope of the present invention.

In the present invention, the term "support" is used broadly as encompassing stents, implants, scaffolding structures and other structures permitting a localized delivery of irradiation. It should be understood that biocompatibility is an important arteria for the support of the invention.

In the following, we discuss two different methods on how to bond the polymer on the stent. The first is to fashion the polymer as a thin film completely encasing the stent. The second is to soak the struts with a thin coating of the polymer.

## EXAMPLE 1

### Encasing film

With this approach, the spatial range of the β particles of $^{35}S$ corresponds to a thick cylinder with an inner and outer diameters of about 2.2 mm and 3.8 mm respectively (figure 2).

Based on symmetry, half the energy is emitted radially inward and half radially outward. The energy emitted inwardly exposes the flowing blood contained in a cylindrical annulus with an inner diameter of 2.2 mm and an outer diameter of 3 mm. Since the exposure time is about 20 days it can be shown that the entire volume of blood passes many times over. As a consequence it can be assumed that the energy emitted inwardly is deposited into a mass of tissue of about 5 Kg (5 litres of blood) and as will be demonstrated below the total dose to the blood is negligible as compared with the small mass of vascular tissue.

Consequently, the dosimetry calculation is performed with the fixed vascular tissue also corresponding to a cylinder annulus with an inner diameter of 3 mm, outer diameter of 3.8 mm and length of 15 mm (stent length) for a mass of about 0.11 g.

In the dosimetry calculations, the radiation is considered as corpuscular radiation. The calculations of the absorbed dose for internally deposited radioisotope follows directly from the definition of the gray (Gy). Over the spatial range (volume) of the considered radiation (β), the absorbed energy should be equal to the concentration of the energy emitted by the radioisotope. The energy absorbed per unit mass per transformation is called the specific effective energy (SEE). For a β particle the SEE is simply the average energy of the radiation divided by the mass of tissue over which it is active (Cember Herman, 1983, Introduction to Health Physics, $2_{nd}$ edition, Pergamon Press, New York).

Since what is proposed is a continuous interstitial radiotherapy (brachytherapy) treatment, a low dose rate delivery is required. Based on previous works in brachytherapy, a total dose of 90 Gy has been adopted (which corresponds to the dose that produces an effect equivalent to 60 Gy in 7 days) (Hall EJ. et al., 1991, Int. J. Radiation Oncology Biol. Phys. 21:1403-1414; Fowler J. et al., 1992, Int. J. Radiation Oncology Biol. Phys. 23:661-669). With a treatment time of 20 days it provides a dose/rate of 0.19 Gy/h which corresponds to a dose/rate known to be effective for human cell lines. It should be understood that the total dose and dose/rate can be adapted by the medical practitioner in accordance with the clinical condition to be treated. In addition, should a γ-emitter or mixed emitter be desired, adaptation of the protocol could be performed by the medical practitioner.

The initial activity on the stent can be related to the dose/rate with the following equation:

$$\dot{D}(\beta)=\frac{q\ Bq\times\frac{tps}{Bq}\times SEE\frac{MeV}{t}}{kg\times 1.6\times 10^{-13}\frac{J}{MeV}\times 8.64\times 10^{4}\frac{sec}{day}}\Bigg/1\frac{J}{kg}\frac{1}{Gy} \qquad [2]$$

combining relations (1) and (2):

$$q(Bq)=\frac{\dot{D}(\beta)\times mkg\times 1\frac{J}{kg}\frac{1}{Gy}}{1\frac{tps}{Bq}\times\bar{E}\frac{MeV}{t}\times 1.6\times 10^{-13}\frac{J}{MeV}\times 8.64\times 10^{4}\frac{sec}{day}} \qquad [3]$$

For this particular application, we assume a constant activity since the physical half life is larger than the biodegradation time of the polymer (a more precise calculation taking into account the decrease in activity can also be performed, however the constant assumption yields a lower initial activity). With the physical characteristics of $^{35}S$ and the considered mass of tissue, it yields:

$$q(Bq)=\frac{4.5\frac{Gy}{day}\times 0.11\times 10^{-3}kg\times 1\frac{J}{kg}\frac{1}{Gy}}{1\frac{tps}{Bq}\times 0.0488MeV\times 1.6\times 10^{-13}\frac{J}{MeV}\times 8.64\times 10^{4}\frac{sec}{day}}=7.338\times 10^{5}Bq \qquad [4]$$

The activity in Curie is computed with:

$$q(Ci)=\frac{qBq}{3.7\times10^{10}\frac{Bq}{Ci}}=\frac{7.338\times10^{5}}{3.7\times10^{10}}Ci=1.98\times10^{-5}Ci=19.8\mu Ci$$

[5]

This initial activity can be assessed using a Beta Counter (liquid scintillator) available at the Montreal Heart Institute. It should be pointed out that the accumulated dose over 20 days of 90 Gy corresponds to an energy of about 0.01 Joule to the vascular tissue, as previously mentioned there should be a similar amount of energy brought to the 5 kg of blood tissue which would mean an additional dose of 2 mGy for the blood tissue and eventually when all the polymer matrix would be dissolved there would be a dose distribution of approximately 0.5 mGy to the cartilage, 0.08 mGy to the bone marrow and 0.005 mGy to the whole body. As a final remark, the calculated activity is within an effective range as previously reported in the literature. It is clear that the use of a modified isotope which enables to be eliminated from the circulation avoids this distribution and provides a further advantage.

It should be noted that the above method requires the polymer to be very elastic. Indeed, for a 3 mm diameter vessel, balloon inflation increases the stent diameter by a factor of two. This implies that the polymer should be capable of sustaining a 100 % deformation without tearing or breaking. This is demanding requirement that limits the possibilities.

## EXAMPLE 2

### Coated struts

A method requiring less stringent elastic properties is presented herein below. The polymer is fixed to the stent struts, the exposed tissues are in the surrounding of the wire as illustrated in figures

3 and 4. As shown, it corresponds to a wire wrapped into a helicoidal shape. When unwrapped and stretched is simply becomes a straight wire. Since $^{35}$ S has a spatial range on about 400 $\mu$m, the mass of the exposed tissues would be $m = \pi r^2 L \rho = 0.005$ L (grams). Where L corresponds to the length of the wire in cm, r the spatial range of the $\beta$ radiation (0.04 cm) and $\rho$_ the density of tissues (~ 1 gram/cm$^3$). A more detailed calculation could be performed taking into account the diameter of the strut and the thickness of the polymer coating.

Again based on symmetry, half the energy is emitted radially inward and half radially outward. Consequently, the mass of fixed vascular tissues would be 0.0025 L (grams) and as in the previous approach the energy emitted radially inward would be deposited into the 5 Kg of blood.

For this approach, the same treatment protocol of 90 Gy in 20 days (0.19 Gy/h) can be proposed. Using the same hypotheses as with the encasing film method, the initial activity can be obtained with the following relation:

$$q(Bq) = \frac{4.5\frac{Gy}{day} \times 0.0025L \times 10^{-3}kg \times 1\frac{J}{kg}\frac{1}{Gy}}{1\frac{fps}{Bq} \times 0.0488MeV \times 1.6\times10^{-13}\frac{J}{MeV} \times 8.64\times10^{4}\frac{sec}{day}} = 1.668\times10^{4}L(cm)Bq \qquad [6]$$

The activity in Curie is given by:

$$q(Ci) = \frac{qBq}{3.7\times10^{10}\frac{Bq}{Ci}} = \frac{1.668\times10^{4}L}{3.7\times10^{10}}Ci = 4.51\times10^{-7}L(cm)Ci = 0.45L(cm)\mu Ci \qquad [7]$$

As in Example 1, the biodistribution can easily be determined by simply substituting the wire length. In this approach, the radiation distribution requires special considerations. Indeed, figures 3 and 4 illustrate that depending on the energy of the β emitter and the proximity of the struts there may be gaps where tissues are not properly exposed to radiation. Such gaps could be filled by using a more energetic β emitter (such as $^{45}$ Ca) or by tightening the stent struts.

$^{45}$ Ca also has a known medical usage. Its maximum and average energies are respectively 257 KeV and 76.2 KeV, corresponding to a spatial range of about 800 µm (twice that of $^{35}$S). The half life of 163 days present the same practical advantages as $^{35}$S.

The use of a biodegradable sheath such as described in 5,383,928 and contains a radioisotope could also circumvent this gap problem.

## EXAMPLE 3

**Animal protocol for evaluating a radioactive stent according to the present invention.**

The objectives of this study is to evaluate the *in vivo* performance of the radioactive heparin-coated Wiktor stent versus non radioactive heparin-coated and uncoated Wiktor stents with respect to its thrombogenicity and neointimal thickening.

It is expected that this study will determine the ability of the radioactive heparin-coated Wiktor stent to reduce: 1) complications of stent thrombosis; and 2) intimal thickening through smooth muscle cells proliferation inhibition.

This study is conducted in a swine model in sub-chronic time frame. Analysis includes quantitative angiography, ultrasonography and histopathology. Controls are non-radioactive heparin-coated and uncoated Wiktor coronary prostheses.

**Test species**

The swine implant model has been previously described with respect to balloon damage, stent placement and biologic response to stents. Male or female (Sus scrofa) of 30-40 kgs are utilized. Each animal is identified with ID number tattooed on the ear or by ear tag as per Montreal Heart Institute standard operating procedures. Stents can be placed in the coronary arteries easily and efficiently. Methods of tissue processing and quantification are also well established.

**Animal health requirements**

These requirements are to be conform to the Montreal Heart Institute standard operating procedures, and in accordance with the National Institute of Health animal research guidelines.

**Exclusions**

Individual swine in poor health or pregnant sows are to be excluded from the study in respect of the Montreal Heart Institute operating procedures, and in accordance with the National Institute of Health animal research guidelines.

**Number of swine in study**

A total of 10 swine are enrolled in this study. After a follow-up of 28+/-2 days, 10 animals are sacrificed. Assuming a long term survival of 80%, 8 swines should thus be available for follow-up analysis.

**Housing, feeding, handling and care of swine**

These aspects of animal care will follow the Montreal Heart Institute standard operating procedures (SOP). Animals are housed per Montreal heart Institute SOP. Animals are fed with standard (non hypercholesterolemic) laboratory food. Precautions are to be observed to avoid potential contaminants present in the food that could influence the results of the study.

All surgical wounds are to be checked regularly by qualified personnel. The wound condition are to be noted on the animals pre and post-operative care sheet.

**Deviations due to condition or disease**

If an animal which has entered the study and has contracted a condition or disease that might interfere with the purpose of the study, that animal will be treated as prescribed by the clinic veterinarian (in accordance with GLP 21 CFR, part 58, subpart E, section 58.90, paragraph C) and the Montreal Heart Institute SOP. The diagnosis, authorizations of treatment, description of treatment and each date of treatment shall be retained.

**Deaths**

The Protocol for termination is described below.

While the number of acute and/or unexpected deaths should be few, if an animal dies before scheduled termination, a necropsy and excision of relevant tissue is to be performed as soon as possible, preferably no longer than 2 hours after death. All information related to the death shall be recorded in the laboratory notebook. The sponsor shall be notified of the death within 24 hours. The animal and tissue are to be treated as described below when possible.

**Test material: Wiktor coronary prosthesis**

Sterilized heparin-coated and uncoated Wiktor stents are used. Stents of 3.5 mm in diameter premounted on usual balloon angioplasty catheter shall also be used. The guidewire used will be 0.014 inches. An 8 French (0.077 " min. ID) guiding catheter (hockey stick or Amplatz) are to be used for all coronary implants.

Each package containing the endovascular prosthesis is identified with a unique serial number. The devices should not require special storage conditions other than expected for a cardiology lab.

**Study personnel Selection**

Selection of staff personnel for this study was carried out according to the "Good Laboratory Practice Regulation" 21 CFR, Part 58, Subpart 8, Section 58.29.

**Surgical methodology**

**Procedure for study**

Information relevant to the surgical procedure of the endoprosthesis are recorded in a laboratory notebook by the designated recorder. At the conclusion of surgery, this person will check that all requested information has been recorded and will sign and date the notebook as per Montreal Heart Institute SOP. The following outlines the approach for the study: 12 animals; Femoral puncture; Stents implants: randomized radioactive heparin-coated, non-radioactive heparin-coated and uncoated in 2 coronary vessels (prox LAD, prox Cx, dist Cx) per animal; each animal will thus contain 3 stents of each characteristics (3 stents/1 pig, balloon/artery ratios ~ 1.2 :1); Adjunctive high pressure inflations (10-12 atm); Angiography, ultrasonography; Sacrifice of the 10 animals at the 4 weeks mark, after angiography and ultrasonography; and Pathology assessment and correlation (quantitative histopathology).

**Drug therapy**

Aspirin is administered the day before treatment, at treatment and every day thereafter for one week. There will be no prolonged systemic anticoagulation followed by chronic warfarin.

**Schedule of angiography and sacrifice post endoprosthesis placement.**

The time points for testing (sacrifice time after stent placement) is be as follows: At 28+/-2 days, sacrifice of the pigs. This is to allow analysis of 10 coronary segments with radioactive heparin-coated Wiktor stents, 10 coronary segments with non radioactive

heparin-coated Wiktor stents and 10 coronary segments with non radioactive uncoated Wiktor stents.

**Presurgical regimen**

Animals are identified, transferred, and weighed in accordance with the Montreal Heart Institute SOP. Hematology and clinical chemistry analyses is performed as indicated by DVM and include ACT, aPTT, TT prior to stent placement.

**Preparation for surgery and anaesthesia**

Intravenous access, anaesthesia, preparation of clinical equipment, and administration of medication is carried out in accordance with the Montreal Heart Institute SOP and accordingly all personnel directly involved in the procedure shall wear appropriate attire.

Preoperative medications is administered one day prior to the balloon injury. Young farm swine (Sus scrofa) weighing 25-30 kgs are given 30 mg nifedipine p.o. 1 day prior to the procedure. Ketamine (12 mg/Kg) and Xylazine (8 mg/Kg im) are administered as a premedication prior to anaesthesia, allowing easy endotracheal intubation. Xylazine is an excellent analgesic and is long acting. Anaesthesia is induced and maintained with 1% halothane in a 1:1 mixture of air or oxygen. A heparin (150 U/Kg) and Xylocaine (100 mg) bolus are administered after percutaneous puncture through the arterial sheath. ACT is measured immediately after administration of heparin and at 30 minutes and 60 minutes thereafter. If the ACT decreases below 300 seconds, an additional 75 U/Kg of heparin will be administered. Saline is administered the day before treatment and during treatment.

**Balloon damage of vessel and stent placement**

Arteries are attributed randomly to receive either one radioactive heparin-coated, one non radioactive heparin-coated or one non radioactive uncoated Wiktor stent. Each artery (LAD, Cx) thus receives one or two stents.

Percutaneous puncture is performed on the right femoral artery, or the carotid artery at the election of the investigator, and the vessel is cannulated with an 8 F vascular sheath. A single bolus of 150 U heparin per Kg and xylocaine (100 mg) is given at this point. An 8 F percutaneous transluminal coronary angioplasty (PTCA) guide catheter is advanced to the aortic root and the left main coronary artery is engaged under fluoroscopic guidance. Nitroglycerin is used as needed to eliminate spasm at final angiographic and ultrasonic assessment of stent deployment.

Each 3.5 mm coronary stent, premounted on the standard PTCA balloon catheter (Gold-X) is used to expand the stent at the intended site. EKGs and blood pressure is continuously monitored during stent implant and post ballooning, and post-procedure, and maintained as per the Montreal Heart Institute SOP. Initial stent deployment is done at 8 atm. Adjunctive high pressure inflations is accomplished using the same Gold-X, semi-compliant balloon whose diameter is equal to 3.5 mm. An inflation pressure of at least 12 atmospheres is to be used. Stent position, expansion and apposition to the vessel wall is then confirmed by IVUS.

After removing the remaining catheters and the introducer sheath, the puncture site is held under pressure by hand until hemostasis is maintained.

QCA analysis will include the minimal in-stent diameter and the proximal and distal vessel diameters (1 cm apart of each end of the stent).

At 28+/- 2 days, 10 pigs are sacrificed. Arteriography and IVUS is performed. At sacrifice, the stented arteries are removed en bloc, along with short (1 cm minimum) artery segments proximal and distal to the stents. All specimens are fixed and evaluated by quantitative histopathology in order to quantify thrombus formation, intimal thickening, and arterial remodelling.

**Follow-up angiography**

Quantitative angiography and intravascular ultrasound are used to assess endoprosthesis patency, placement and apposition to the arterial wall.

Computerized digital angiographic image analysis (View System, Baxter Inc.) is performed with the guiding catheter used as reference. In the event that the coronary endoprosthesis is occluded and the animal has survived, the animal will be sacrificed according to the termination protocol.

**Termination protocol**

Animal sacrifice is to be conducted in 10 animals at 28+/-2 days, or in the event the animal is under stress and deemed to be at risk of death. An intravenous commercial solution can be given by intravenous administration for euthanasia (e.g. "Sleepaway", Fort Dodge Laboratories, 10 cc) as per the Montreal Heart Institute SOPs.

General physical examination includes body weight and general condition of animal and character of wound healing. Laboratory examination analyses the complete blood count as warranted by DVM, the Serum chemistry as warranted by DVM and ACT, aPTT, and TT immediately pre-sacrifice. Samples for these tests are to be obtained prior to administration of any anticoagulants or drugs required for angiography or sacrifice.

**Animal sacrifice**

Heparin (approximately 150 U/Kg) is to be administered intravenously and the animal is then be sacrificed.

**Necropsy examination**

Necropsy examination is to be performed by the designated pathologist A cardiovascular gross examination is done. This is an external examination of the body including all organs in the thoracic cavity. Tissues and organs observed include the mesenteric lymph

nodes, lungs and main stem bronchi, heart, aorta and vessels of the left upper leg. The heart is examined with respect to potential emboli. Additional examination of other tissues and organs, e.g. the brains will be done if considered relevant by the clinical investigators or the veterinary pathologist.

Gross morphology assessment is performed by taking a macroscopic photography of the necropsied heart to show any gross response to stent implant.

All vascular endoprostheses are examined in situ, explanted and photographed. Photographs will be labelled with study centre, swine number, date, description of tissue photographed and technician's initials.

The vessels segment with the stent is excised from the heart. Explanted vascular prosthesis tissue is to include the entire endoprosthesis and at least 1 cm of native artery attached to each end of the prosthesis. The proximal arterial end is to be marked by the insertion of a black ligature and the distal end by insertion of 2 black ligatures. The appearance of the proximal and distal arterial ends and mid-prosthesis sections is to be described.

Native vessel proximal and distal to the explanted segment are also examined. Photographs of the intact explanted endoprosthesis are to be taken, including the entire explanted segments as well as close-ups of the proximal and distal arterial ends and mid-endoprosthesis region.

Samples will be pressure fixed in the MJK/2 fixative and will undergo histological preparation and evaluation as described in the section above.

All explants samples and specimens are to be labelled with the study centre, animal number, the coronary vessel in which it was implanted, date of explant, and description of the sample.

Handling and disposal of animal carcasses is to be conducted following the Montreal Heart Institute SOPs.

**Tissue histopathology**

**Tissue handling, fixation and staining**

All sections are fixed in MJK/2 (formula given below) under physiologic pressure. This allows samples to be viewed by SEM if desired. The vessel with the stent is removed so that 1 cm of vessel protrudes beyond the ends of the stent. The sample is cut in cross-sections so that the stent and artery are cut in 2 equal lengths. The distal segment is used for histological analysis. Transverse sections are cut at 5 mm intervals starting at the central portion and moving to the distal segment (marked with a ligature). Histopathologic analysis must include sections at the centre of the stent; at the distal edge of the stent with the vessel wall and at the vessel wall 5 mm from the edge of the stent. The diameter of the lumen proximal, distal, and in the stented area is measured. Evaluation of outer diameter enlargement, filling defect, patency of side branches, protrusions of the stent into the vessel lumen, medial or adventitial cells modifications or fibrosis, and neointimal thickening around artery circumference is recorded in the pathology report. Any signs of inflammatory reactions against stents struts is documented.

Histological strains include: H&E, Masson's Trichrome, Elastic von Giesson stains. Slides are to be obtained for quantitative histopathological analysis.

**MJK/2 Fixative**

20 gm paraformaldehyde

250 ml $H_2O$

Heat but do not boil (60 to 70°c) and stir constantly. Add 10-40 drops of 1N NaOH while stirring to clear solution.

Cool solution with running cold water.

Add: 50 ml of 50% glutaraldehyde.

400 ml of O.2 M Cacodylate buffer (formula below)

300 ml of distilled water

Adjust pH to 7.2 to 7.4

Add 50 mg of CaCl2- (100 drops) of 1% solution

Refrigerate

**Cacodylate buffer**

42.8 gm Sodium cacodylate

1000 ml of D.D. water

Add 9-10 ml of 1 N HCl to bring it up to a pH of 7.2-7.4

Cacodylate buffer for washing tissue after MJK/2 fixation

50 ml of 0.2 M cacodylate buffer

50 ml of D.D. water

5 gm of sucrose

**Quantitative histopathology**

The artery sections is observed with quantitative low and high power light microscopy where a movable calibrated reticule is utilized for making lengths measurements in the plane of microscopic view. Careful estimation of the luminal thickening around the arteries circumference of each of the histological sections is made. Quantitative and qualitative findings are to be recorded.

Histomorphometry

After specific preparation, using embedding in epoxy-resins, stented arteries are cut into slices with a rotating diamond saw. Morphometric analysis should include: maximal intimal thickness, arc length of the medial fracture, lumen area, neo-intima perimeter and total vessel perimeter. The ratio of intimal thickness to length is to be calculated to correct for the extent of the vessel injury.

If needed, some arteries segments can be examined by scanning electron microscopy in order to assess the reendothelialization of the luminal surface.

Immuno-histology

To visualize smooth muscle cells and endothelial cell populations, immuno-staining is performed. Specific monoclonal antibodies against proliferative nuclear antigen (PCNA) can assess the limitation of the smooth cell proliferation.

Throughout this application, various publications are referenced. The disclosure of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

The preceding examples are intended to illustrate but not limit the invention. While they are typical of those that might be used, other procedures known to those skilled in the art may be alternatively employed.

Although the present invention has been described herein above by way of preferred embodiments thereof, it can be modified, without departing from the spirit and nature of the subject invention as defined in the appended claims.

WHAT IS CLAIMED IS:

1. A radioactivity local delivery system comprising:

a)a support of generally tubular structure having an external surface adapted to engage the wall of a vessel of a human patient;

b)a radioisotope in releasable association with said support; and

c)a chelating agent coupled to said radioisotope,

whereby upon placement of said radioactivity local delivery system inside said vessel, said releasable association between said support and said radioisotope enables a release of said radioisotope thereof into the circulation of said human patient at a rate controlled by the rate of release of said association, and said chelating agent enables a rapid elimination of said radioisotope from said circulation.

2. The radioactivity local delivery system of claim 1, wherein said support is selected from a group consisting of a scaffolding structure, a stent and a body implant.

3. The radioactivity local delivery system of claim 1 or 2, wherein said support is comprised of a biodegradable material into which said radioisotope is embedded such that said releasable association between said radioisotope and said support is provided by said biodegradable substance.

4. The radioactivity local delivery system of claim 1 or 2, wherein said support is in contact with a coating substance comprising said radioisotope.

5. The radioactivity local delivery system of claim 4, wherein said coating substance is selected from the group consisting of a natural biodegradable substance, a synthetic biodegradable substance and a non-biodegradable substance.

6. The radioactivity local delivery system of claim 1, 2, 3, 4 or 5, wherein said rate of release of said association enables a control of said local delivery of radiation.

7. The radioactivity local delivery system of claim 1, 2, 3, 4, 5, or 6, wherein said radioisotope is selected from a group of pure beta-emitter, pure gamma-emitter and mixed beta- and gamma-emitter.

8. The radioactivity local delivery system of claim 1, 2, 3, 4, 5, 6, or 7, wherein said support is a stent adapted for angioplasty, said radioisotope is a pure beta-emitter and said dose of radiation is between 20 Gy and 200 Gy.

9. The radioactivity local delivery system of claim 8 wherein said dose of radiation is approximately 60 Gy in 7 days equivalent.

10. The radioactivity local delivery system of claims 1, 2, 3, 4, 5, 7, 8, 9 wherein said radioisotope is $^{45}Ca$ coupled to a chelating agent.

11. The radioactivity local delivery wherein said chelating agent is selected from a group consisting of EDTA, DTPA and DFO.

12. A kit comprising a support of generally tubular structure having an external surface adapted to engage the wall of a vessel of a human patient and a radioisotope coupled to a chelating agent, wherein said radioisotope is in releasable association with said support for locally delivering to a targeted site of a human vessel a predetermined dose of radiation.

13. A cell proliferation inhibiting composition comprising a radioisotope coupled to a chelating agent, wherein said radioisotope is in releasable association with a pharmaceutically acceptable carrier, whereby, upon placement of said cell proliferation inhibiting composition at a targeted site in a human patient, said irradiation by said radioisotope inhibits a proliferation of actively proliferating cells and whereby said releasable association between said radioisotope and said carrier enables a controlled release of said radioisotope from said targeted site at a controlled rate into the circulation of said patient and said chelating agent enables a rapid elimination of said radioisotope from said circulation.

14. A method to decrease the growth of actively proliferating cells at a targeted site in a vessel of a human patient comprising steps of inserting and positioning at said site of a composition consisting of a radioisotope coupled to a chelating agent, wherein said radioisotope is in releasable association with a pharmaceutically acceptable carrier; such that irradiation inhibits a proliferation of said actively proliferating cells and such that, by way of said releasable association between said radioisotope and said carrier, said radioisotope is removed from said targeted site at a controlled rate into the circulation

of said patient and said chelating agent enables a rapid elimination of said radioisotope from said circulation.

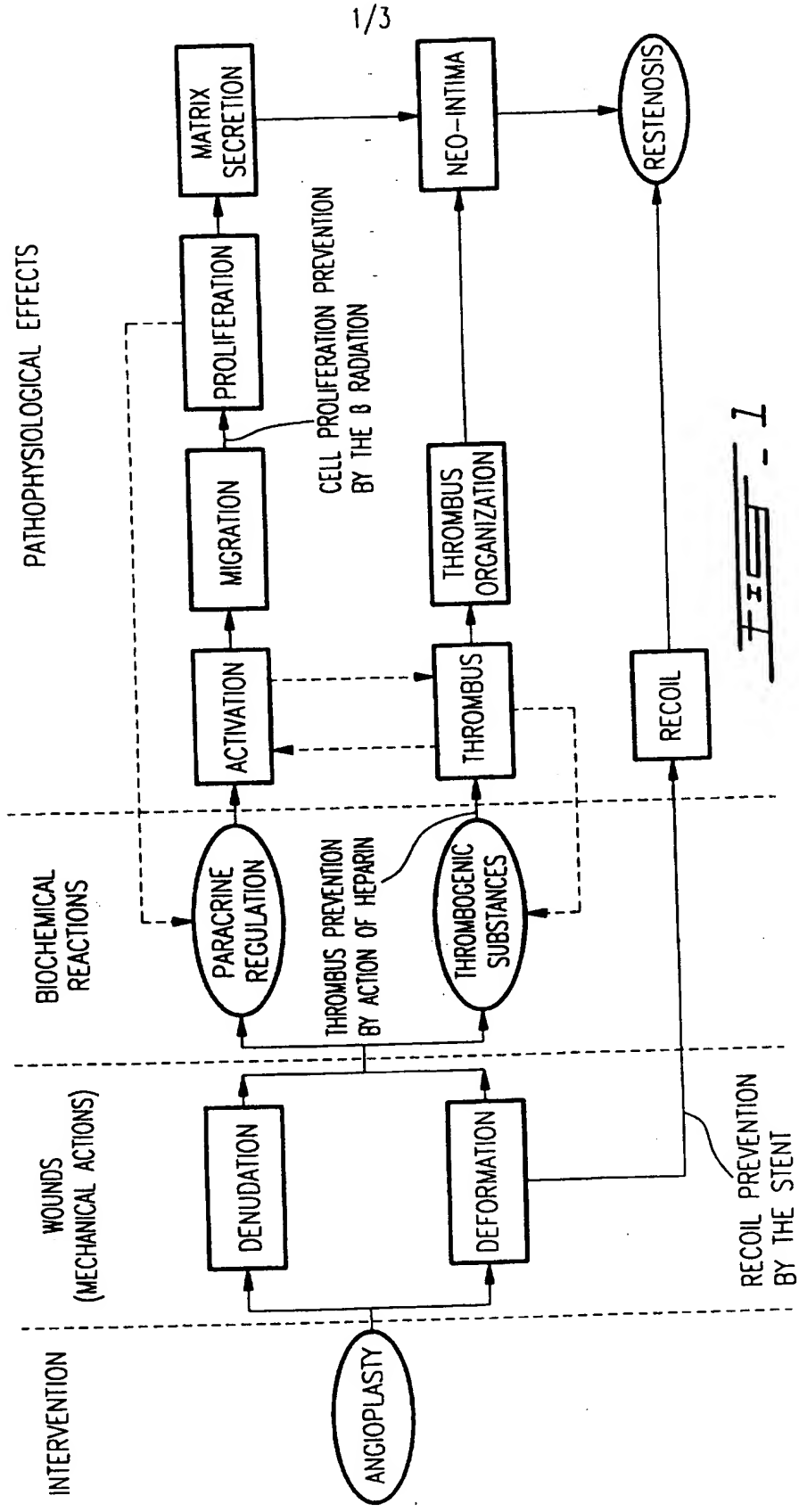
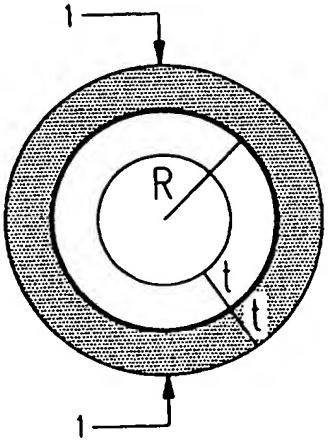
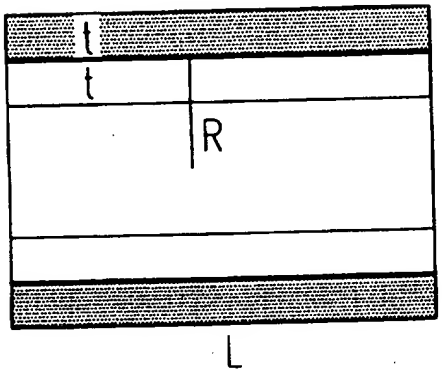



Fig - 1



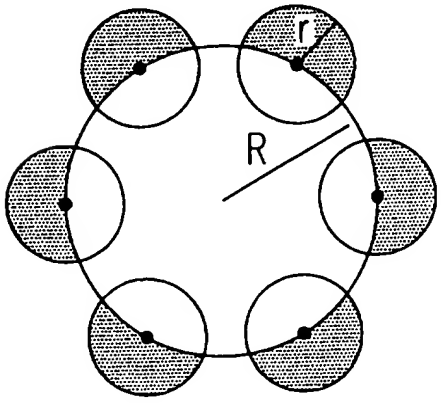

R : Vessel radius (1.5 mm)
t : Spatial range of radiation (0.4 mm)
L : Vessel segment (stent) length (15 mm)
▨ : Fixed vessel tissue exposed to radiation
□ : Flowing blood exposed to radiation

Fig. 2A




R : Vessel radius (1.5 mm)
t : Spatial range of radiation (0.4 mm)
L : Vessel segment (stent) length (15 mm)
▨ : Fixed vessel tissue exposed to radiation
□ : Flowing blood exposed to radiation

Fig. 2B




r : Spatial range of radiation (0.4 mm)
R : Vessel radius (1.5 mm)
• : Stent struts
□ : Flowing blood exposed to radiation
▨ : Fixed vessel tissue exposed to radiation

Fig. 3

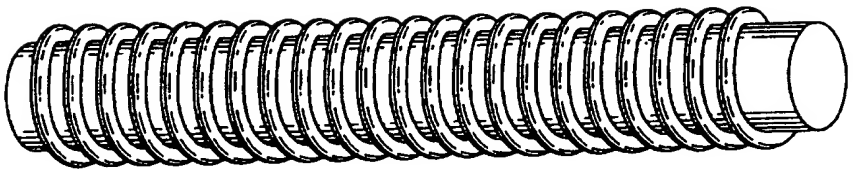


FIG. 4A

FIG. 4B

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(57) Abstract

The invention provides a radioactivity local delivery system enabling the use of isotopes having a longer half life and having a lower energy. The invention also provides a radioactivity local delivery system enabling an easier and more efficient control of the dose/rate and total dose of local radiation delivery. The present invention utilizes a radioisotope coupled to a circulation time reducing agent such as a chelatin agent, the coupled radioisotope being in releasable association with the structure to be inserted in a vessel of a human patient. The coupled radioisotope can be part of the support itself or in releasable association therewith through the use of a biodegradable or non-biodegradable pharmaceutical carrier. Also provided is a method to decrease the growth of actively proliferating cells at a targeted site in a vessel of a human patient as well as compositions therefor.

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# INTERNATIONAL SEARCH REPORT

Inte. onal Application No
PCT/CA 97/00262

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K51/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Berte, M |

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# INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 97/ 00262

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This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

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This International Searching Authority found multiple inventions in this international application, as follows

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